



Centers for Disease Control
and Prevention (CDC)
National Center for Infectious Diseases
1324 Calle Cañada
San Juan, Puerto Rico 00920-3860
Tel. (787) 706-2399
FAX (787) 706-2496

Criteria for the processing of dengue samples at the CDC Dengue Branch, San Juan, Puerto Rico

The diagnosis and treatment of dengue and dengue hemorrhagic fever are guided by the symptoms and findings that the patient presents, and cannot depend on laboratory confirmation, since there routine tests can not confirm dengue with the speed required for patients in critical condition. The processing of samples for serologic diagnosis takes approximately one week, and the virus isolation takes from one to three weeks. Even so, it is necessary to eventually have a confirmation of the diagnosis, to exclude other etiologic possibilities, and to guide the follow-up of the patient's convalescence.

To obtain correct data on the spread of disease, adequately decide what laboratory tests to use, correctly interpret test results, and to assure that results get to the person who requested them, the following information is indispensable:

- Complete name, age and sex of the patient
- Home address
- Date of onset of symptoms
- Date that sample was obtained
- Complete name and mailing address of the physician, laboratory, clinic or hospital that the result should be sent to.

Samples without the above-mentioned information, or written with illegible handwriting, or with more than a month from date of sample collection to date of arrival at CDC, will not be analyzed.

The Dengue Branch of the Centers for Disease Control and Prevention (CDC) can process and report the results of a limited number of samples per week. These samples are of great importance to determine the serotype and genetic make-up of viruses being transmitted in the region, and to determine their geographic distribution. This information can be used to detect risk factors that may lead to a new and more severe pattern of disease, and to refine the clinical

diagnostic ability of attending physicians. Diagnostic bleeding is not provided to persons referred to CDC.

Epidemic Response

In case of a severe dengue epidemic, CDC Dengue Branch will promptly analyze samples received with the minimum above-mentioned information. They will be analyzed in the following order of priority:

- fatal cases,
- cases in intensive care,
- hospitalized cases (with thrombocytopenia, hemorrhage, shock or hemoconcentration),
- all other cases.

In epidemic periods, the samples that have insufficient priority for processing in that week's batch, will be set aside for later analysis when the Laboratory returns to its routine functioning. In such periods, the physician, laboratory or clinic will receive results with one or two months' delay. Those samples without any of the priority conditions stated above, and which, due to the volume of priority samples, are not analyzed within a month, will be eliminated from the analysis queue.

We want to emphasize that to maintain efficient dengue surveillance, we will continue processing samples from outpatients and mildly ill persons any time of the year, and especially when dengue incidence in the island is relatively low (usually during the months from April to July).

We will appreciate that you consult with us (Dr. Mark Beatty or Dr. Jorge Muñoz) about any doubt or problem related to these criteria, to maintain open communication among the Laboratory and the physicians, clinics, and laboratories, and to continue the mutual collaboration that has been very fruitful throughout the years.

Case Notification and Shipment of Samples of Suspected Dengue Cases

Dengue is characterized by an acute febrile picture accompanied by headache, retroorbital pain, body pain, often a rash, and other variable symptoms that can include obvious or mild hemorrhagic manifestations (such as a petechial rash) or hemoconcentration, shock or coma. This disease should be considered whenever there is an increase in the number of persons who go to the physician with an acute febrile illness, complaints of "monga" or "influenza," or an increase in the number of clinically diagnosed cases of German measles or regular measles.

Instructions for obtaining and handling samples:

1. Once there is a clinical diagnosis of suspected dengue, take a blood sample (see #4 and #5) and fill out the Dengue Case Investigation Form (see copy attached). **With this Form you comply with the legal reporting requirement.** These forms can be obtained from the CDC Dengue Branch and also from the Regional Environmental Health Office of the Puerto Rico Department of Health. They can also be photocopied without restriction.
2. It is of great importance to fill out the Dengue Case Investigation Form in a clear and complete manner. The information received on each case (especially the date of onset of symptoms and date of sample collection) is crucial to select and interpret the laboratory analyses. Furthermore, a complete address makes it possible to identify the area where control measures should be implemented. **Samples without the above-mentioned information, or written in illegible handwriting or with more than a month from date of collection to date of arrival at CDC, will not be analyzed.**
3. The blood sample is taken in a red-top tube (preferably, but if not, you can use a green-top tube). Violet-top tubes (with heparin) should not be used. After separating the serum, it must be maintained on ice or in a refrigerator (not in a freezer) until it is delivered to CDC Dengue Branch. The case investigation forms and the acute blood sample should reach CDC Dengue Branch as soon as possible. They can be sent through the local Environmental Health office. The acute sample can be sent immediately; there is no need to wait until the convalescent sample is taken.
4. To diagnose dengue, the laboratory requires a blood sample taken during the acute period of the disease and a second sample that can be taken from day 6 after the onset of symptoms. Informing the patient about the importance of coming back for a second sample, and giving an appointment for a specific day and hour, will increase the probability of obtaining the second sample. If the patient makes the first visit to the physician on or after day 6 after onset of the symptoms, that sample is enough. In that case, it is not necessary that the patient come for a second sample.
5. Acute-phase samples (taken on or before day 3 after onset of symptoms), will be used for virus isolation by mosquito cell cultures or by *Toxorhynchites amboinensis* or *Aedes aegypti* mosquito inoculation. Convalescent-phase samples (taken on or after day 6 after beginning of symptoms) will be used for identification of IgM or IgG anti-dengue antibodies by enzyme-linked immunosorbent assays (ELISA).

<u>Type of sample</u>	<u>Interval since the onset of symptoms</u>	<u>Type of Analysis</u>
"Acute"	until day 3	Virology
"Convalescent"	6 or more days	Serology

Samples taken on days 4 and 5 of illness are of low yield for isolation as well as serology. Samples obtained those days will be held for analysis only if a second sample is received.

WHENEVER THERE IS A HOSPITALIZED SEVERE CASE, PLEASE INDICATE IT IN THE CASE INVESTIGATION FORM.

6. Reports will be sent to the physician (if the return address has been indicated) with the results of positive, or clearly negative, cases. In cases with negative virus isolation, we will await a convalescent-phase sample before reporting a result.
7. Results will be reported only to the laboratory or the physician who sent the sample (or an authorized secretary).

Dengue Branch, CDC
1324 Calle Cañada
San Juan, PR 00920-3860

Tel. (787) 706-2399

CHECK LIST FOR OBTAINING AND SHIPPING DENGUE DIAGNOSTIC SAMPLES

[] Sample

Type of sample	Interval since date of onset of symptoms	Type of Analysis
----------------	---	---------------------

Acute	up to 3 days	Virology
Intermediate	4 or 5 days	*
Convalescent	6 or more days	Serology

* Depends on the result of the convalescent serum.

[] **Form** - "Dengue Case Investigation Form"

Can be obtained from the CDC Dengue Branch in San Juan.

Please indicate on the sheet if the case is hospitalized. If it is a very severe case, indicate so on the "Comments" section.

Only the samples received with the information requested below, and written in a legible manner, will be analyzed:

- Complete name, age, and sex of patient
- Home address
- Date of onset of symptoms
- Date sample was obtained
- Complete name and mailing address of the physician, laboratory, clinic, or hospital

[] **Tube** - Red or green top (not violet).

[] **Labeling** - Tube and case form must agree (indicate the same name of the case).

[] **Volume** - 2 cc. (ml.) of centrifuged serum or plasma

[] **Storage** - On ice or in a refrigerator (not in a freezer) until it is delivered to the CDC Dengue Branch.

[] **Time of shipment** - Not to exceed a month after taking the sample

[] **Way of shipment** - Check with local Department of Health.

Reasons for **REJECTING** samples:

Samples without form, form without sample

Incomplete or illegible form – especially regarding date of onset of symptoms, date of sample collection

Hemolyzed or frozen sample, or received more than a month after onset of illness

Dengue Branch, CDC

1324 Calle Cañada

San Juan, PR 00920-3860

Tel. (787) 706-2399

G:\NEWS\TestpolEng.doc



DENGUE CASE INVESTIGATION REPORT
CDC Dengue Branch and Puerto Rico Department of Health
1324 Calle Cañada, San Juan, P. R. 00920-3860
Tel. (787) 706-2399, Fax (787) 706-2496



For CDC Dengue Branch use only									
GCODE		Specimen #	Days post onset (DPO)	Type	Received (Date)	Specimen #	Days post onset (DPO)	Type	Received (Date)
SAN ID		S1			/ /	S3			/ /
		S2			/ /	S4			/ /

Please complete all sections

Hospitalized: <input type="checkbox"/> No <input type="checkbox"/> Yes Hospital:	Fatal: <input type="checkbox"/> Yes <input type="checkbox"/> No	Encephalitis: <input type="checkbox"/> Yes <input type="checkbox"/> No
--	---	--

Name:

Last Name First Name Middle Name / Initial

If a minor, name of parent or person in charge:

Home Address	Physician who referred the case:
City, Town:	Name:
Urbanization or sector:	Phone number:
Street: Number:	Send results to:
Premise No.: Box: P.O.Box:	
Road No.: Km: Hm: Tel.:	
Close to:	

Patient's Basic Information	Additional Data
Date of birth: Age: Sex: <input type="checkbox"/> Male <input type="checkbox"/> Female	1) Country of birth:
years	2) Have you had dengue before (fever, body pain, eye pain, rash) <input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Don't know
Day Month Year	3) When? (Month, Year) / <input type="checkbox"/> No <input type="checkbox"/> Don't know

Indispensable information for sample processing	4) How long have you lived in this city?
Date of first symptom: Day Month Year / /	5) During the 14 days before onset of illness, have you traveled to other cities or countries? <input type="checkbox"/> yes <input type="checkbox"/> no <input type="checkbox"/> don't know
Date specimen taken	Where?
Serum: first sample illness. / / (acute - first 5 days of sickness - for virus)	
second sample / / (convalescent - 6 or more days after sickness - for antibodies)	
third sample / /	
Other tissue: / /	

Criteria for DENGUE HEMORRHAGIC FEVER (#1- 4) and shock (#5)

1. Fever ☐ yes ☐ no

2. Any hemorrhagic manifestation

Petechiae ☐ yes ☐ no
Purpura/Echymosis.. ☐ yes ☐ no
Vomit with blood..... ☐ yes ☐ no
Blood in stool..... ☐ yes ☐ no
Nasal bleeding..... ☐ yes ☐ no
Bleeding gums..... ☐ yes ☐ no
Blood in urine..... ☐ yes ☐ no
Vaginal bleeding..... ☐ yes ☐ no
Urinalysis - over 5 RBC/hpf or positive for blood.... ☐ yes ☐ no
Tourniquet test _not done _Pos _Neg

3. Platelets $\leq 100,000/\text{mm}^3$.. ☐ yes ☐ no

(count) _____

4. Leaky capillaries

Pleural or abdominal effusion.. ☐ yes ☐ no

Lowest hematocrit _____

Highest hematocrit _____

Lowest serum albumin _____

Lowest serum protein _____

5. Lowest blood pressure _____ / _____

Other symptoms

Headache ☐ yes ☐ no

Eye pain ... ☐ yes ☐ no

Body pain ☐ yes ☐ no

Joint pain..... ☐ yes ☐ no

Rash ☐ yes ☐ no
Chills ☐ yes ☐ no
Nausea o vomiting ... ☐ yes ☐ no
Diarrhea ☐ yes ☐ no
Cough ☐ yes ☐ no
Conjunctivitis ☐ yes ☐ no
Nasal Congestion ☐ yes ☐ no
Sore throat ☐ yes ☐ no
Jaundice..... ☐ yes ☐ no
Convulsion or coma.. ☐ yes ☐ no
Pregnant?..... ☐ yes ☐ no
YF vaccination..... ☐ yes ☐ no
year _____ ☐ doesn't know

FOR CDC DENGUE BRANCH USE ONLY

Specimen No.

S¹ _____

S² _____.

S³ _____

SEROLOGY

Hemagglutination Inhibition

Test	Ag	Titer	Test	Ag	Titer	Test	Ag	Titer

IgG Antibody

Test	Ag	Qual	Titer	Test	Ag	Qual	Titer	Test	Ag	Qual	Titer

IgM Antibody

Test	Ag	Value	Test	Ag	Value	Test	Ag	Value

Neutralization

Test	Ag	Titer	Test	Ag	Titer	Test	Ag	Titer

VIROLOGY

Test	ID	Isotech	IDtech	Test	ID	Isotech	IDtech	Test	ID	Isotech	IDtech

Overall interpretation: